Aroma Volatile Biosynthesis in Apples at Harvest or After Harvest Affected by Jasmonates

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Keywords: apples, ethylene, jasmonates, volatile

Abstract

Effects of jasmonates on production of aroma volatile compounds by 'Tsugaru' apples [*Malus sylvestris* (L.) Mill. var. domestica (Borkh.) Mansf.] at harvest, and by 'Delicious' apples after harvest were examined. Among volatiles classified as alcohols, esters, ketones, aldehydes, acetic acid, and α-farnesene, esters were the most prevalent compounds, followed by alcohols. Jasmonate treatment at pre-climacteric stage increased the production of esters (butyl propanoate, butyl butyrate, and propyl butyrate) and of anthocyanin. In addition, jasmonate treatment stimulated 1-aminocyclopropane-1-carboxylate oxidase 1 (*ACO 1*) mRNA transcript and thylene production at 3 days after treatment. This result suggests that jasmonates may influence ACC oxidase activity. The impact of jasmonate application after harvest on volatile production differed with cultivar. The combination of ethephon with jasmonates reduced volatile production by 'Delicious' compared with ethephon only. The effect of jasmonates on volatile production was related to the effect of jasmonates on internal ethylene concentration. The results show that the effect of jasmonates on aroma volatiles in apples may be mediated by ethylene. Furthermore, the effect of jasmonates on aroma volatiles may depend on the fruit development stage when treated with jasmonates.

INTRODUCTION

Aroma volatile compounds are one of the factors that determine apple fruit quality. Aroma production increases with ripening and is associated with ethylene production (Song and Bangerth, 1996; Lalel et al., 2003b). Methyl jasmonate (MeJA) also influences the production of volatile compounds, but the effect can vary with volatile compound chemical class (Fan et al., 1997). Although many types of aroma volatiles are synthesized in apple fruit, the relative abundance of each type differs among cultivars (Fan et al., 1997; Mattheis et al., 1998). For most cultivars, esters are qualitatively and quantitatively predominant (Rowan et al., 1999). Aroma volatiles are primarily synthesized in the skin (Knee and Hatfield, 1981), and Kondo et al. (2001) showed that jasmonates effectively stimulate anthocyanin biosynthesis and that the effect is independent of ethylene. Therefore, jasmonates have been applied prior to harvest in the field for the promotion of red color development in Japan. However, the effect of jasmonate applied prior to harvest on aroma volatiles is unclear. In this study, effects of jasmonates and ethephon, alone or in combination, on aroma volatile and ethylene production during apple fruit ripening were examined.

MATERIALS AND METHODS

Chemicals

n-Propyl dihydrojasmonate (PDJ, a.i. 5.0 %) was a gift from Nippon Zeon Co. (Tokyo). 2-Chloroethyl phosphonic acid (Ethephon, a. i. 21.7 %) was purchased from Rhone-Poulenc Co. (Research Triangle Park, NC). Methyl jasmonate (MeJA, 95 %) was purchased from Sigma-Aldrich Co. (Milwaukee, WI).

Plant Material

Six randomly selected 17-year-old 'Tsugaru' apple trees, grafted onto Malling 9 (M. 9) rootstocks, growing in an open field at Prefectural University of Hiroshima were used in 2005. Each tree was trained as a central leader and planted in a single row from east to west with spacing of 3.0 m × 4.0 m. Furthermore, for 'Tsugaru', PDJ solution of 0.39 mM was applied by spraying whole trees 107 days after full bloom (DAFB). Thirty fruit (10 from each tree) were sampled at 7 days intervals. For 'Delicious' fruit were harvested 179 DAFB in 2003. Immediately after harvest, 304 fruit were randomly separated to 4 groups of 76 fruit for the following treatments; 1) MeJA, 2) Ethephon, 3) Ethephon + MeJA, 4) Untreated control. Group 1 and 3 fruit were dipped for 5 min in a solution of 0.177 % (v/v) Tween® 20 with 5 mM MeJA or the same solution in combination with 400 a. i. $mg \cdot L^{-1}$ ethephon, respectively. Group 4 fruit were dipped for 5 min in a solution of the same concentration of Tween® 20 in deionized water (untreated control). After treatment, apples were placed in the dark at 20 °C and sampled at 7 days intervals for analysis of volatile compounds, ethylene production, and jasmonate concentrations.

Analysis of Aroma Volatile Compounds and Ethylene

Analysis of volatile compounds was performed with a modification of the method previously described by Mattheis et al. (1991). Four apples per treatment (three replications) were placed in four 1 glass jars and the jars purged with air that had been passed through activated charcoal and molecular sieve. Air flowed through the jars at 50 ml min^{-1} for 3 h before collection of volatile compounds onto glass traps containing 50 mg Tenax GC (Allteh Assoc., Deerfield, IL). The absorbed contents in the trap were desorbed into the injection port of gas chromatograph with a mass selective detector (GC-MSD) (HP 5890, 5971A, Hewlett Packard, Avondale, PA). The column oven temperature was held at 35°C for 3 min, then increased from 35°C to 225°C at a rate of 8°C min^{-1}. For 'Tsugaru', hue value and ethylene production from fruit were measured according to the previous report (Kondo and Takano, 2000; Kondo et al., 2001). For 'Delicious', internal ethylene in the core was analyzed, using a HP5890 gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a glass column (610 mm x 3.2 mm i.d.) packed with Porapak Q (80-100 mesh) (Supelco, Bellefonte, PA) and flame ionization detector.

Extraction and Analysis of Jasmonates

Extraction and quantification of MeJA were carried out as described previously by Kondo et al. (2000). The deuterium-labeled MeJA [($^2H_2$)-MeJA: methyl (±)-[9, 10-$^2H_2$] jasmonate] used for the internal standard was prepared according to the method of Seto et al. (1996). Lyophilized skinless pulp samples (5 g dry weight (DW)); three replications) were homogenized with 1 µg ($^2H_2$) MeJA in 50 ml diethyl ether containing 11.3 µM butylated hydroxytoluene as an antioxidant, 20 ml saturated NaCl solution, and 1 ml 1M citric acid. The MeJA in the samples was derivatized with pentafluorobenzyl (PFB) bromide. The amount of PFB-MeJA was analyzed by GC-MS selected ion monitoring (SIM) (QP 5000; Shimadzu, Kyoto, Japan).

Northern Blot Hybridization

cDNA of *ACC oxidase (ACO 1)* was a gift from Dr. T. Harada at Hirosaki university. The fragment used for probes was from bp 3558 to bp 3780 of the *ACO 1* with accession number AF030859. The Northern blot analysis was carried out using previously reported method (kondo et al., 2002).

Statistical Analysis

The SAS ANOVA procedure was used to determine treatment effects, and mean separation was analyzed by Fisher's least significant difference ($p \leq 0.05$)(SAS, Cary, NC).

RESULTS

PDJ treatment at 107 DAFB increased ethylene, total alcohol, and total ester production in 'Tsugaru' apples (Fig. 1). However, aldehyde production was not different between PDJ and untreated control. PDJ also increased the accumulation of *ACO 1* mRNA at 3 and 6 days after treatment (Fig. 2). For 'Delicious', untreated control and ethephon-treated fruit had high internal ethylene concentrations (Fig. 3). Ethylene concentrations in MeJA-treated fruit were second or third lowest, and the combination with ethephon decreased the levels compared with ethephon treatment only. Forty-four volatile compounds in 'Delicious' were detected. These volatiles were of six groups: alcohols (1-butanol, ethanol, 1-hexanol, 2-methyl-1-butanol, 2-methyl-1-propanol, 1-propanol, and 2-propanol), esters (butyl acetate, butyl butyrate, butyl hexanoate, butyl 2-methylbutyrate, butyl propanoate, ethyl acetate, ethyl butyrate, ethyl 2-methylbutyrate, ethyl hexanoate, ethyl propanoate, ethyl octanoate, hexyl acetate, hexyl butyrate, hexyl hexanoate, hexyl 2-methylbutyrate, hexyl propanoate, 2-methylbutyl acetate, methyl butyrate, 2-methylbutyl-2-methylbutyrate, methyl 2-methylbutyrate, pentyl acetate, propyl acetate, propyl butyrate, propyl propanoate, and 2-methylpropyl acetate), ketones (acetone and 6-mehyl-5-heptan-2-one), aldehydes (benzaldehyde, butanal, decanal, 2-furancarboxaldehyde, hexanal, heptanal, nonanal, octanal, pentanal, and 4-allylanisole), acetic acid, and α-farnesene.

Ester and alcohol production levels were higher than those of any other volatile group. Ethanol and 1-propanol were primary volatiles in the alcohol group, and butyl acetate, ethyl acetate, hexyl acetate, 2-methylbutyl acetate, and propyl acetate were foremost in the ester group. Alcohol and ester production increased with days ripening (DR) toward 28 DR (Fig. 4). The combination of ethephon and MeJA decreased alcohol and ester production compared with ethephon treatment only. MeJA treatment only also decreased alcohol and ester production.

Ketone and aldehyde production increased slightly during storage (Data not presented). Treatment with ethephon increased production of these compounds. The combination of MeJA and ethephon decreased production compared with ethephon only. However, MeJA and ethephon accelerated ketone production. Although acetic acid production in untreated controls or MeJA-treated fruit was high at 14 DR, no increases with DR were observed (Data not presented). α-Farnesene production by untreated controls and ethephon-treated fruit increased until 14 DR but then decreased (Data not presented).

DISCUSSION

PDJ, which is a jasmonic acid derivative that shows stable effects in the field compared with MeJA (Fujisawa et al., 1997), has been applied to promote anthocyanin accumulation before harvest in apples. It has been reported that MeJA application increased aroma volatiles in mangos (Lalel et al., 2003a). PDJ treatment prior to harvest also stimulated aroma volatiles in apples.

Volatile compounds in apples, produced by lipid and amino acid catabolism, are primarily synthesized in the skin (Fan et al., 1197; Rudell et al., 2002). Palmitic acid, stearic acid, oleic acid, linoleic acid, and triacontane were detected as the main lipids in apple skin at harvest, although the levels of melissic acid, montanic acid, and heptacosan were high in immature fruit skin (Noro et al., 1985). Thus, it is assumed that the late-forming lipids are associated with aroma volatile synthesis during fruit ripening. Ethephon application increases production of aroma volatiles and lipid concentrations in mangoes (Lalel et al., 2003b). The effect of MeJA on internal ethylene concentration and production of volatile compounds differed among cultivars. MeJA decreased internal ethylene concentrations in 'Delicious' compared with the untreated control. MeJA treatment of pre-climacteric apple fruit enhances ethylene production; in contrast, ethylene production in climacteric apple fruit is reduced by MeJA treatment (Saniewski et al., 1986). Although the mechanism is unclear, the effect of MeJA on ethylene production may be via ACC synthase or ACC oxidase because MeJA influences enzyme activity such

as UDP-glucose flavonoid 3-*O*-glucosyltransferase, lipase, and protease (Ranjan and Lewak, 1995; Kondo, 2003). This hypothesis is supported from the result that *ACO 1* mRNA transcript was stimulated by PDJ treatment (Fig. 2). It seems that 'Delicious' apples were harvested after the onset of the climacteric based on the effect of MeJA on internal ethylene concentrations (Kondo et al., 2001). This result in our study shows the effect of MeJA on aroma volatiles depends on the development stage of the treated fruit. MeJA may decrease aroma volatiles when applied at the middle of the climacteric stage. However, from the correlative relationship between internal ethylene and volatiles such as alcohols and esters, MeJA application at preclimacteric stage may stimulate volatile production.

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Figures

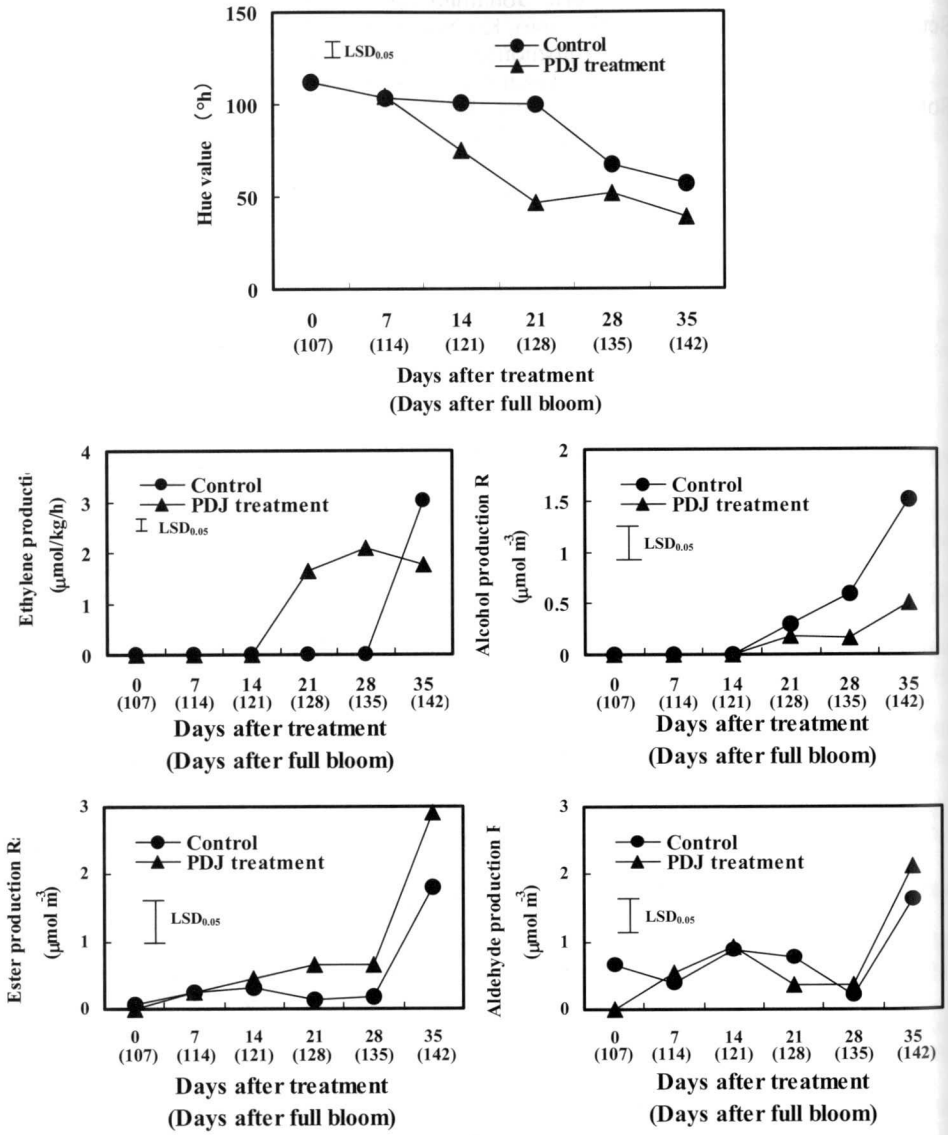

Fig. 1. Effects of PDJ treatment prior to harvest on hue value, ethylene, total alcohol, total ester, and total aldehyde in 'Tsugaru' apples.

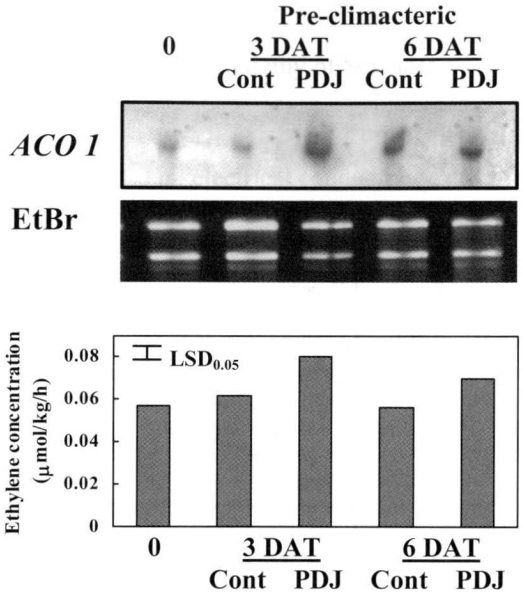

Fig. 2. Effects of PDJ treatment on ACC oxidase gene expression and ethylene concentration in the skin of 'Tsugaru' apples. PDJ solution was sprayed the fruit at 114 days after full bloom. DAT: Days after treatment.

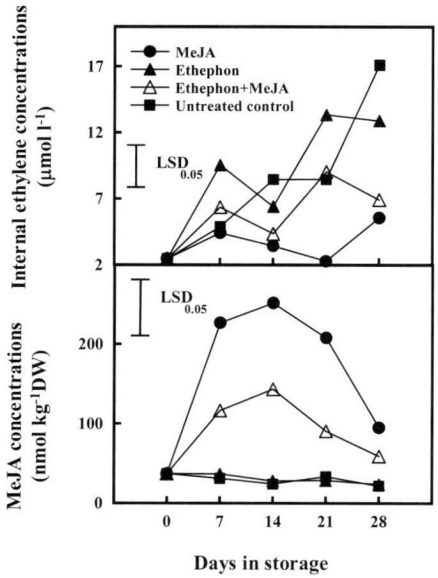

Fig. 3. Effects of MeJA and ethephon on internal ethylene and MeJA concentrations in 'Delicious' apples.

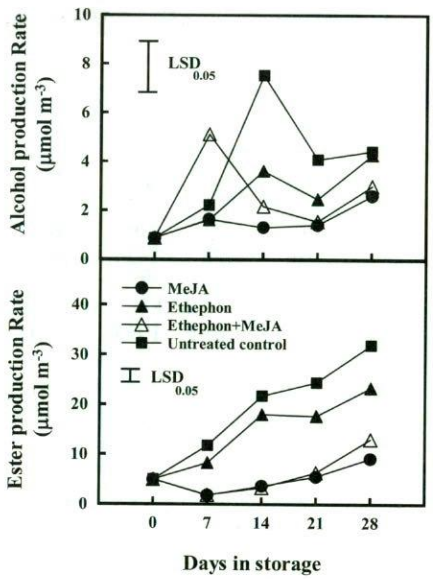

Fig. 4. Effects of MeJA and ethephon on total alcohol and total ester production in 'Delicious' apples.